REMARKS

The claims have been amended to refer to "very early stage" breast cancer.

Support for this amendment can be found, *inter alia*, at page 6, line 18 of the present specification, which refers to breast cancer; page 7, first full paragraph which refers to the identification of stages of disease which may be identified in accordance with the present invention; and, in particular, page 11, line 16 which refers to detection at "very early stages" of the disease.[1/]

In paragraph 3, on page 2 of the Office Action, the Examiner rejects Claims 18-35 under 35 U.S.C. § 112, first paragraph as failing to meet the written description requirement.

Specifically, the Examiner states that the specification does not provide support for using blood cells in a patient with breast cancer, where the blood cells have not contacted the area of disease.

In addition, in paragraph 4, on page 3 of the Office Action, the Examiner rejects Claims 18-35 under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement.

Specifically, the Examiner state that the specification does not provide guidance on how to identify cells in a blood

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[1/] Ralph et al and Zhi-Xin et al, previously relied upon by the Examiner, relate to detection of metastatic cancers, and thus in no way relate to "very early stage" cancers, much less, "very early stage breast" cancer, as presently claimed.

sample that have "not come into contact with the area of said cancer".

The Examiner notes Applicants' argument that the prior art shows that it was known to isolate blood cells that have not come into contact with a breast cancer, i.e., such would occur with breast cancer tumors that have not yet extended beyond the basement member into the surrounding stoma, i.e., they have not penetrated into the circulatory system. However, the Examiner states that the claims are not limited to such breast cancers. Further, the Examiner states that while Applicants have provided Appendix A with the Amendment filed February 8, 2007, to demonstrate that the present invention is effective to diagnose ductal carcinoma *in situ* (DCIS) and IDC (stage I cancer), the claims are not so limited, and thus cover any breast cancer or stage thereof.[2/]

In paragraph 5, on page 12 of the Office Action, the Examiner rejects Claims 27-35 under 35 U.S.C. § 112, first paragraph.

Specifically, it is the Examiner's position that the method of the claimed invention requires hybridization to a particular set of probes that are identified only by their function and by the type of cell that were identified within. However, it is the Examiner's position that the specification fails to describe a single probe useful in the present invention, and thus the claims lack written description.

[2/] Applicants note, the Examiner also criticizes Appendix A as not being in Declaration format. A Declaration under 37 C.F.R. § 1.132 is submitted herewith meeting the Examiner's objection.

In view of the amendments to the claims, Applicants respectfully submit that the Examiner's rejections have been rendered moot.

Specifically, the claims have been limited to "very early stage" breast cancer. Very early stage breast cancer is also known in the art as stage 0 breast cancer (see the attached executed Declaration under 37 C.F.R. § 1.132). Such cancers are breast cancers that are restricted to milk ducts and have not yet extended beyond the basement member into the surrounding stoma, i.e., they have not penetrated into the circulatory system.

While in the present invention, the probes are isolated from very early stage breast cancer patients, the probes can be used to diagnose a variety of breast cancer patients. That is, the very early stage breast cancer probes can be used to diagnose stage I or II breast cancer patients (for example) as having breast cancer.

As previously discussed, DCIS patients (i.e., stage 0 patients) could readily be identified at the time of the present invention. Thus, it is a matter of routine to identify very early stage breast cancer patients and to isolate their blood. These samples could thus be obtained reproducibly and reliably. This aspect is thus fully enabled.

Furthermore, Applicants have also already provided evidence that DCIS (stage 0) obtained probes can be used to diagnose DCIS and stage I cancer. This was provided in the above-discussed Appendix A. As noted above, this evidence is now provided in the form of the attached Declaration under 37 C.F.R. § 1.132.

Specifically, Figures 2B and 2C of the Declaration use stage 0 breast cancer probes and show that stage I and stage 0 breast cancer samples, respectively, can be successfully diagnosed.

The Examiner alleges that the method which is performed in Appendix A (now the Declaration) is not a method described in the instant specification, i.e., the specification teaches away from using hybridization to pre-selected probes for the identification of markers, particularly since the specification refers to the exclusion of non-sequence-based separation techniques.

The Examiner is requested to note that the use of non-sequence-based methods of probe identification is only one method according to the specification. The application as filed is directed to two separate embodiments. For example, on page 10, last two paragraphs; on page 23, second full paragraph; and page 15, lines 11-14 of the specification, one embodiment of the present invention is described, which relates to the use of samples, particularly blood, which are distant to the area of disease, e.g., a tumor, to identify differentially expressed transcripts which may then be used for diagnostic purposes. The various advantages of these non-invasive techniques are described in the sections mentioned above. The use of non-sequence-based separation techniques is clearly unrelated to this embodiment of the present invention as described in the specification, as it is clear that there is no requirement to select the probes in a particular way. Instead, the invention lies in the samples to be used.

The use of non-sequence based techniques for the separation of probes in methods of the present invention constitutes a second embodiment described in the specification, see for example page 13, lines 20 *et seq.*, which refers to the advantages which flow from the use of that technique.

Thus, clearly two embodiments are described in the present specification, the first being directed to the identification of probes from a specific sample source, and the second being concerned with the identification of probes by using non-sequence based separation techniques. The claims as now presented are concerned with the first embodiment and do not include the use of a non-sequence based separation technique. Thus, the results presented in Appendix A (now the Declaration) are obtained in accordance with methods as presented in the specification, and are in line with the present claims.

The Examiner also alleges that the methods can not be reliably performed because one can not reliably obtain probes suitable for diagnosis from the thousands of possible genes, that the different levels of expression do not necessarily associate with a diagnosis, that the thresholds of variation in expression required have not been established, that 2 individuals would not provide enough information and that data analysis which was not taught in the specification has a major effect on the result.

The Examiner is requested to note that the invention does not lie in the particular probes that are isolated, but rather in the samples which are used. Thus, essentially any very early stage breast cancer probes which show differential expression,

of which there are many, can be used. The Examiner has acknowledged that the level of skill in differential gene expression is high.

The present application teaches that one can look in blood samples to identify transcripts which are differentially expressed in very early stage breast cancer versus normal patients. This teaching is sufficient to perform the invention. One can readily obtain such samples, and methods to obtain differentially expressed transcripts by comparing transcripts from different samples was well-known in the art, as acknowledged by the Examiner. In each comparison, the skilled person would reliably obtain differentially expressed transcripts which can be used for diagnostic purposes. The sequence of the probes is not of importance. The relevant issue is instead whether suitable probes can be obtained. While in different experiments, different sets of differentially expressed probes might be obtained, in each case, when these probes are used diagnostically, they successfully allow the identification, and hence diagnosis of breast cancer samples. The different levels of expression found in the transcripts successfully allows diagnosis to be performed.

Thus, the Declaration simply provides an illustration of what would be observed when putting the teachings of the present invention into effect. Routine differential expression analysis reveals a set of probes which can then be used in predictive diagnostic methods. The comparative methods which are used are routine and could be put into effect at the time of the invention. Thus, the data in the Declaration shows that the

present invention is fully enabled for the skilled person to identify probes which can be used diagnostically according to the present invention.

With regard to the Examiner's comments on the use of just two organisms to generate the data, whilst this may not be the preferred number of organisms to be used for comparative purposes (this is set as the minimum), two is sufficient. It is, of course, standard to eliminate variables, such as age and gender, when comparing samples such that if only two organisms are used they are matched as far as possible to remove sources of variability other than their disease state. Indeed on page 35 of the present specification, in referring to the development of a reproducible expression pattern, it is indicated that the patient to be diagnosed with the disease of interest relative to a normal patient should be matched as far as possible with regard to age, sex and other factors. Page 15 of the present specification describes in general what is considered by differential expression and refers to this being established between normal and disease samples; thus, it is clear that any other interferences must be eliminated. Furthermore page 21 of the present specification teaches that it is appropriate to use multiple samples to obtain the patterns. Page 22, last paragraph of the present specification, also refers to obtaining standard deviations for several representative samples, as opposed to the use of single samples. Thus, it is clear that the potential problem of using non-matched individuals is recognized and one is taught how this should be addressed. In some cases, two organisms may be

sufficient for comparative purposes. This is the theoretical minimum in which two samples from the diseased class and two from the healthy class would be used. At least two samples must be present to calculate the variance in gene expression in the disease and non-disease classes.

The Examiner has also commented on the threshold of variation in expression required to establish a gene expression difference and the mode of data analysis which is not taught in the specification and which would have a major effect on the results obtained.

Such information is not provided in the specification, as these aspects were well-known in the art at the time of the present invention. Thus, the specification legally need only provide relevant information to supplement that already known to the skilled person.

Several statistical tools were available in the prior art at the time of the present invention which could be used to analyse multivariate data. In fact, Partial Least Square Regression Analysis, the technique Applicants normally use for model building (SDPP development) and prediction is described in Martens et al, *Multivariate Calibration*, Chichester etc., Wiley (1989); and Höskuldsson, *J. Chemometrics.*, 2(3):211-228 (1988); a copy of each of which is attached hereto. Even when the sample size is small, results can be efficiently validated using the cross-validation approach as described in the Declaration. This approach was described as early as 1982 (Efron, "The Jackknife, the Bootstrap and Other Resampling Plans", *Society*

for Industrial and Applied Mathematics, Philadelphia, Pennsylvania (1982); a copy of which is attached hereto).

Thus, the methods of discriminating between informative and non-informative probes, i.e., setting a threshold for determining if "differential" expression exists and for analyzing data, was well-known in the art. Following the methods as taught in the art with the samples as taught in the specification (and claims) would reliably yield diagnostic methods according to the claims. The analytical methods which would be used were routine at the time of the present invention. Thus, the present invention could be readily put into practice by the skilled person at the time of the present invention. Hence, no specific example is required.

In the Declaration, the breast cancers were divided by stage (stage 0 or I) rather than by type. Thus, in the case of stage I different types of breast cancers were included. The results showed that 80% of the stage I samples were correctly predicted using the stage 0 probes. Hence, in the absence of any concrete evidence that the present invention would not be suitable to diagnose all types of breast cancer, the evidence as provided is sufficient to show enablement for the present claim scope.

Accordingly, Applicants respectfully submit that the claims clearly have written description support in the specification and are enabled by the present specification. Thus, Applicants request withdrawal of the Examiner's rejections.

In paragraph 7, on page 14 of the Office Action, the Examiner provisionally rejects Claims 15-38 under the doctrine

of obviousness-type double-patenting as being unpatentable over Claims 1-36 of co-pending application, Serial No. 11/149,370.

As this rejection is provisional in nature, i.e., the co-pending application has not yet issued into a patent, this rejection is traversed on that basis, i.e., once the remaining rejections are overcome, this rejection should be withdrawn.

In view of the amendments to the amendments to the claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at the below-listed number on any matters which might arise.

Respectfully submitted,

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